Technology
Biomyx
Innovation & Convenience for Life Sciences

Biomyx Technology
10054 Mesa Ridge Court, Suite 112
San Diego, CA 92121
Phone: (858) 457-3658 FAX: (858) 457-3485
www.biomyx.net

P53 ProtéGene™ Set

Catalog# P1035
Lot# 171021

Materials Provided

1. pMEV-P53-WT (P1035a): 20 μg in 40 μl TE (pH7.5), 0.5 mg/ml.
2. pMEV-P53-DN (P1035b): 20 μg in 40 μl TE (pH7.5), 0.5 mg/ml.
3. Product Information Sheets.

Note: *Individual plasmids can be ordered separately. Some plasmids are shipped as lyophilized pellet.*

Receiving and Storage:

If received in liquid form, spin the vials briefly in a microcentrifuge to collect the contents. If received in lyophilized form, add 40 μl sterile DI water to the vial, mix thoroughly by vortex and then collect the contents by centrifuging the vials briefly in a microcentrifuge. Store the products at 2-8^{0}C if used immediately and store at –20^{0}C for extended storage.

Expression Vector:

pMEV-2HA (a): Cat# P1001a.

Affinity Tag:

N-terminal 2 x HA, a 9-aa peptide derived from influenza virus (MGYPYDVPDYAYPYDVPDYAGS…) .

Prokaryotic Selection:

The kanamycin-resistance gene (aminoglcoside 3' phospho-transferase) expression cassette in the plasmids confers Kanamycin resistance to bacteria cells. Bacterial cells transformed with the plasmids should be maintained and grown in media containing 25-50μg/ml Kanamycin (e.g. #LK-1100, Prepured LB Agar plates, Biomyx, San Diego, California).

Eukaryotic Selection:

The neomycin resistance gene, driven by SV40 early promoter, confers G418 resistance to eukaryotic cells. Stable mammalian cell lines can be selected with G418.

Description of P53 and Mutants

The p53 protein is a nuclear phosphoprotein of 53 kDa. It is a tumor suppressor protein critical for regulation of the cell cycle in response to genotoxic insults such as radiation or chemicals resulting in DNA damages. The highly conserved vertebrate p53 gene is one of the most frequently mutated genes found in human cancers, being either lost or mutated in over half of all human tumors See Cox and Lane, 1995; Levine, 1997 for review). It has therefore become the center of intensive study ever since the link of p53 mutations with various human cancers was realized. The expression level of several important genes including p21, MDM2, GADD45 (CHOP), Bax and cyclin G was modulated by p53. Among many other roles, it is involved in arresting the cell cycle at G1 phase upon DNA damages (Cox and Lane, 1995; Levine, 1997).

The native p53 protein is a tetramer in solution and functions as a transcription factor that binds to specific DNA sequences. Many P53 mutations, especially those lie within the DNA-binding domain (so-called hot-spot mutations), show dominance over wild type protein. Replacement of valine 143, one of the hot-spot residues, results in a mutant (V143A) with a transdominant-negative effect on several activities of wild type p53. V143A is also a temperature-sensitive mutant: it shows DNA binding and transcriptional activity at 32°C, but it is inactive at 37°C (see Wong et al., 1999; Bartussek et al., 2002, for example).

Molecular Features of the Inserts:

Gene: *Homo sapiens* tumor protein p53 (Li-Fraumeni syndrome) (TP53)
GenBank Reference Sequence: NM_000546
5'-Cloning Site: Bam HI
5'-Junction Sequence(upper strand) :
5'-…tac gct gga tcc **ATG GAG GAG**…3'
3'-Cloning Site: Eco RI
3'-Junction Sequence (lower strand):
5'-…acgcgtgaattc **TCA GTC TGA GTC** …-3'

P53 Protein Sequence

(393 amino acid residues. V143 is in bold and underlined.)

```
MEEPQSDPSVEPPLSQETFSDLWKLLPENNVLSPLPSQAMDDLMLSPDDI
EQWFTEDPGPDEAPRMPEAAPRVAPAPAAPTPAAPAPAPSWPLSSSVPSQ
KTYQGSYGFRLGFLHSGTAKSVTCTYSPALNKMFCQLAKTCPVQLWVDST
PPPGTRVRAMAIYKQSQHMTEVVRRCPHHERCSDSDGLAPPQHLIRVEGN
LRVEYLDDRNTFRHSVVVPYEPPEVGSDCTTIHYNYMCNSSCMGGMNRRP
ILTIITLEDSSGNLLGRNSFEVRVCACPGRDRRTEEENLRKKGEPHHELP
PGSTKRALPNNTSSSPQPKKKPLDGEYFTLQIRGRERFEMFRELNEALEL
KDAQAGKEPGGSRAHSSHLKSKKGQSTSRHKKLMFKTEGPDSD
```

P53 Nucleotide Sequence

(1182 bps. Codon for V143 is in bold and underlined)

```
   1  ATGGAGGAGC CGCAGTCAGA TCCTAGCGTC GAGCCCCCTC TGAGTCAGGA
  51  AACATTTTCA GACCTATGGA AACTACTTCC TGAAAACAAC GTTCTGTCCC
 101  CCTTGCCGTC CCAAGCAATG GATGATTTGA TGCTGTCCCC GGACGATATT
 151  GAACAATGGT TCACTGAAGA CCCAGGTCCA GATGAAGCTC CCAGAATGCC
 201  AGAGGCTGCT CCCCGCGTGG CCCCTGCACC AGCGACTCCT ACACCGGCGG
 251  CCCCTGCACC AGCCCCCTCC TGGCCCCTGT CATCTTCTGT CCCTTCCCAG
 301  AAAACCTACC AGGGCAGCTA CGGTTTCCGT CTGGGCTTCT TGCATTCTGG
 351  GACAGCCAAG TCTGTGACTT GCACGTACTC CCCTGCCCTC AACAAGATGT
 401  TTTGCCAACT GGCCAAGACC TGCCCTGTGC AGCTGTGGGT TGATTCCACA
 451  CCCCCGCCCG GCACCCGCGT CCGCGCCATG GCCATCTACA AGCAGTCACA
 501  GCACATGACG GAGGTTGTGA GGCGCTGCCC CCACCATGAG CGCTGCTCAG
 551  ATAGCGATGG TCTGGCCCCT CCTCAGCATC TTATCCGAGT GGAAGGAAAT
 601  TTGCGTGTGG AGTATTTGGA TGACAGAAAC ACTTTTCGAC ATAGTGTGGT
 651  GGTGCCCTAT GAGCCGCCTG AGGTTGGCTC TGACTGTACC ACCATCCACT
 701  ACAACTACAT GTGTAACAGT TCCTGCATGG GCGGCATGAA CCGGAGGCCC
 751  ATCCTCACCA TCATCACACT GGAAGACTCC AGTGGTAATC TACTGGGACG
 801  GAACAGCTTT GAGGTGCGTG TTTGTGCCTG TCCTGGGAGA GACCGGCGCA
 851  CAGAGGAAGA GAATCTCCGC AAGAAAGGGG AGCCTCACCA CGAGCTGCCC
 901  CCAGGGAGCA CTAAGCGAGC ACTGCCCAAC AACACCAGCT CCTCTCCCCA
 951  GCCAAAGAAG AAACCACTGG ATGGAGAATA TTTCACCCTT CAGATCCGTG
1001  GGCGTGAGCG CTTCGAGATG TTCCGAGAGC TGAATGAGGC CTTGGAACTC
1051  AAGGATGCCC AGGCTGGGAA GGAGCCAGGG GGGAGCAGGG CTCACTCCAG
1101  CCACCTGAAG TCCAAAAAGG GTCAGTCTAC CTCCCGCCAT AAAAAACTCA
1151  TGTTCAAGAC AGAAGGGCCT GACTCAGACT GA
```

Mutations:

pMEV-P53-WT (P1035a): No mutation
pMEV-P53-DN (P1035b): V143A (GTG to GCG)

Selected References:

Tang C-H and Grimm E, 2004. Depletion of Endogenous Nitric Oxide EnhancesCisplatin-induced Apoptosis in a *p53*-dependent Manner in Melanoma Cell Lines. J. Biol. Chem. 279:288-298

Bartussek C, Naumann U, Weller M. 2002. Tumour p53 mutations exhibit promoter selective dominance over wild type p53. Oncogene. 21:1641-8

Cox., L.S. and Lane, D.P., 1995, Tumor suppressors, kinases and clamps: how p53 regulates the cell cycle in response to DNA damage. BioEssays, 17: 501-508

Kern, S.E., Pietenpol, J.A., Thiagalingam, S., Seymour, A., Kinzler, K.W., and Bogelstein., B., 1992, Oncogenic forms of p53 inhibite p53-regulated gene expression. Science, 256: 827-830

Levine, A., 1997, p53, the cellular gate keeper for growth and division. Cell, 88:323-331

Wong KB, DeDecker BS, Freund SM, Proctor MR, Bycroft M, Fersht AR., 1999. Hot-spot mutants of p53 core domain evince characteristic local structural changes. Proc Natl Acad Sci USA. 96: 8438-42

Zakut-Houri, R., Bienz-Tadmor, B, Givol, D. and Oren, M., 1985. Human p53 cellular tumor antigen: Cdna sequence and expression in COS cells. The EMBO J., 4: 1251-1255

Biomyx Technology
10054 Mesa Ridge Court, Suite 112
San Diego, CA 92121
Phone: (858) 457-3658 FAX: (858) 457-3485
www.biomyx.net

Zambetti, G. P., Bargonetti, J., Walker, K., Prives, C. and Levine, A. J., 1992, Wild-type p53 mediates positive regulation of gene expression through a specific DNA sequence element. Gene & Dev. , 6: 1143-1152